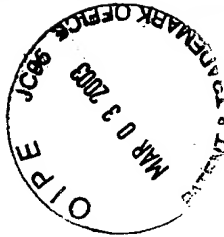


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REMARKS

Status of the claims

Claims 32 and 33 are under consideration in this application and both claims stand rejected. After entry of the amendments made herein, claim 34 will be under consideration, claims 32 and 33 having been cancelled without prejudice to their being presented in a separate application and claim 34 having been added.

New claim 34 contains limitations from cancelled claims 32 and 33 but, in addition, specifies that delivery at a CNS or PNS lesion site is directly at the lesion site. This amendment is supported by the specification, e.g., the text at page 28, lines 15-16, which states in relevant part: "To apply C3 to crushed nerves, Gelfoam soaked with 2 mg/ml C3 was wrapped around the left optic nerve at the crush site, . . ." (emphasis added)

None of the amendments made herein add new matter.

Specification and Drawings

As requested in paragraph 6 of the Office Action, an abstract has been added to the application.

In addition, the specification and the drawings have been amended in accordance with the comments on page 3, lines 9-20, of the Office Action. Typographic errors have also been corrected.

Claim objections

Applicants respectfully submit that the comments on page 4 are moot in light of the cancellation of claims 32 and 33.

35 U.S.C. §112, first paragraph, rejection

Claims 32 and 33 stand rejected on the grounds that they allegedly contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention.

Applicants respectfully submit that the rejection is moot in view of the cancellation of claims 32 and 33.

35 U.S.C. §102(e) rejections

Claims 32 and 33 stand rejected as allegedly being anticipated by Liao et al. and Johnson et al.

Applicants respectfully submit that the rejection is moot in light of the cancellation of claims 32 and 33.

With respect to claim 34, Applicants point out that neither of the cited references teaches a method of suppressing the inhibition of neuronal axon growth by delivering C3 directly at a CNS or PNS lesion site in a patient, i.e., neither of the references specifically teaches such a localized delivery to a CNS or PNS lesion.

The Examiner indicates that Liao et al. relates to the use of a substance for increasing endothelial cell NOS activity. However, the passages mentioned by the Examiner do not specifically teach a localized delivery of C3 for suppressing the inhibition of neuronal axon growth. Thus, for example, the mention of the administration of a substance *in vivo* does not specifically teach the delivery of a substance directly at a lesion site in a person for suppressing the inhibition of neuronal axon growth.

In addition, the examiner indicates that Johnson et al. relates to the use of a substance for regulating actin polymerization, stress fiber formation, and/or focal adhesion assembly. However, the passages mentioned by the examiner with respect to Johnson et al. do not specifically teach a localized delivery of C3 for suppressing the inhibition of neuronal axon growth. Thus, for example, the mention of the administration of a substance systemically does not specifically teach the delivery of a substance directly at a lesion site in a person for suppressing the inhibition of neuronal axon growth. Furthermore, Applicants respectfully submit that the addition of a substance to a cell in vitro (see Examples 1, 2 and 3 of Johnson et al.) is not the same as delivery of a substance directly at a lesion site in a person for suppressing the inhibition of neuronal axon growth.

Applicants respectfully submit that, in light of the above considerations, claim 34 is not anticipated by either of the cited references.

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Attached is a marked-up version of the changes being made by the current amendment.

CONCLUSION

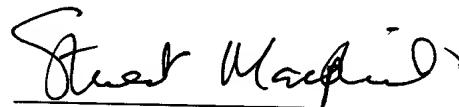
In summary, for the reasons set forth above, Applicants maintain that claim 34 patentably defines the invention. Applicants request that the Examiner reconsider the rejections as set forth in the Office Action, and permit claim 34 to pass to allowance.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicants' undersigned representative can be reached at the telephone number listed below.

Enclosed is a Petition for an Extension of Time with the required fee. Please charge any other fees or make any credits to Deposit Account No. 06-1050, referencing Attorney Docket No. 12552-002001.

Respectfully submitted,

Date: 2/26/03



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Version with markings to show changes made

In the specification:

Paragraph beginning at page 6, line 22 has been amended as follows:

--Fig.[ure] 1 shows results of treatment with C3 to stimulate neurite outgrowth on inhibitory MAG substrates. [A)] Fig. 1A shows PC12 cells plated on MAG remained rounded and did not extend neurites. [B)] Fig. 1B shows [C)] cells plated on MAG in the presence of C3 grew neurites. [C)] Fig. 1C shows PC12 cells plated on polylysine (PLL) substrates as a positive control.--

Paragraph beginning at page 7, line 4, has been amended as follows:

--Fig.[ure] 2 shows the role of integrins in overriding growth inhibition by myelin. The anti- $\alpha 1$ integrin function blocking antibody, 3A3, was used to determine if integrin function is necessary for laminin to override growth inhibition by myelin or MAG. For experiments on myelin substrates (Figs. 2A-D), cells were fluorescently labeled with DiI, and plated on myelin (Fig. 2A), polylysine (Fig. 2B), or myelin +1 μ g laminin (Figs. 2C and D). Control IgG was added to samples shown in Figs. 2A-C, the 3A3 antibody to the sample shown in Fig. 2D. Neurites do not extend on myelin but grow on laminin or mixed laminin/myelin substrates. When 3A3 is added, laminin no longer overrides growth inhibition by myelin. [Panels] [(] Figs. 2E-H[()] show by phase contrast cells plated on recombinant MAG (Fig. 2E), laminin (Fig. 2F), or recombinant MAG plus laminin (Figs. 2G and H), with control antibody (Figs. 2E-G) or with 3A3 (Fig. 2H). Integrin function is needed to override growth inhibition by MAG.--

Paragraph beginning at page 7, line 14, has been amended as follows:

--Fig.[ure] 3 presents the results of studies in which PC12 cells transfected with dominant negative Rho extend short neurites on MAG substrates. Mock-transfected PC12 cells ([a,c,e] Figs. 3A, C, and E) or cells transfected with dominant-negative Rho ([b,d,f] Figs. 3B, D, and F) were plated on laminin ([a,b] Figs. 3A and B) or MAG ([c-f] Figs. C-F). MAG inhibits neurite outgrowth ([c] Fig. 3C), but dominant negative Rho cells spread on MAG and some cells extend

short neurites ([d] Fig. 3D). Treatment with C3 further stimulates neurite outgrowth on MAG from both lines of cells ([e,f] Figs. 3E and F).--

Paragraph beginning at page 7, line 20, has been amended as follows:

--Fig.[ure] 4 shows activation of Rho on MAG substrates. Activated Rho is associated with the plasma membrane. To determine if activated Rho was detected under conditions where PC12 cells do not grow neurites, cells were grown in suspension or plated on MAG or collagen substrates. Two hours later the plasma membranes were purified, the proteins separated by SDS PAGE, and the proteins transferred to nitrocellulose and stained with Ponceau S (top panel). Rho A was detected on the blots by immunoreactivity with anti-RhoA antibody (bottom panel). Immunoreactivity was strongest when cells were grown in suspension or when cells were plated on MAG. Therefore, Rho A is more active when cells are kept in suspension or plated on MAG than when plated on growth-permissive collagen.--

Paragraph beginning at page 8, line 2, has been amended as follows:

--Fig.[ure] 5 shows treatment of retinal neurons with C3 stimulates neurite growth on polylysine and MAG substrates. On nMAG substrates neurite growth is inhibited ([a] Fig. 5A), but after C3 treatment retinal neurons plated nMAG substrates extend neurites ([b] Fig. 5B). Growth of neurites from retinal neurons plated on PLL ([c] Fig. 5C). Bar, 50 μ m.--

Paragraph beginning at page 8, line 6, has been amended as follows:

--Fig.[ure] 6 demonstrates ADP-ribosylation of Rho by C3 detected in cultured cells. PC12 cells or retinal neurons were cultured in the presence (+) or absence of C3 (-) for two days. The cells were lysed, and 10 μ g of protein from each sample was separated on a 11% acrylamide gel. The proteins were transferred to nitrocellulose, probed with mouse anti-RhoA antibody and anti-mouse-HRP antibody, and revealed by a chemiluminescent reaction (top panel). The membranes were then reprobbed with rabbit anti-Cdc42 and anti-rabbit alkaline phosphatase and revealed with NTB/BCIP color reaction (bottom panel). Treatment of cells with C3 result[s] in an ADP-ribosylation-induced decrease in the mobility of RhoA. The mobility of Cdc42 does not change with C3 treatment.--

Paragraph beginning at page 8, line 14, has been amended as follows:

--Fig.[ure] 7 illustrates methods used to study the effect of C3 on injured optic nerve.

Figure 7a shows the optic nerve was removed from the sheath prior to crushing with 10.0 sutures (top panel) and C3 was applied in Gelfoam and Elvax tubes ([red] rectangular bars in middle and bottom panels) immediately following optic nerve crush (middle panel). The retinal ganglion cell axons were detected by anterograde labeling with cholera toxin and immunodetection of the cholera toxin in longitudinal sections of the optic nerve (bottom panel). Fig[ure]s. 7[c, 7d, 7e, and 7f]C-F show treatment of crushed optic nerve with C3 stimulates regenerative growth of retinal ganglion cells axons. ([c] Fig. 7C) Longitudinal 15 μm section of a buffer-treated control optic nerve showing the failure of RGC axons to cross the injured region; (Figs. 7D and E [d,e]) Longitudinal 15 μm sections of two different optic nerves treated with C3 showing anterogradely-labeled axons extending past the crush (arrows). The site of crush is indicated with arrowheads; ([f] Fig. 7F) Higher magnification view [of (e)] in Fig. 2E showing the twisted growth of regenerating axons. Bar, 100 μm ([c,d,e] Figs. 7C-E) and 50 μm in [f] Fig. 7F. Fig.[ure] 7[b]B shows quantitation of axon regeneration across the site of lesion. Representation of regeneration observed in different animals. For each animal, the maximum number of axons observed in a single 14 μm section was counted at different distances from the site of the crush. Each point represents one animal, but animals with growth past 500 μm are also represented at the shorter distances. Large numbers of regenerating fibers (>10/section) were observed to cross the lesion after C3 treatment compared to treatment with PBS.--

Paragraph beginning at page 27, line 11, has been amended as follows:

--To test the involvement of Rho in the response of primary neurons to MAG and to myelin substrates, we purified retinal neurons and treated them with C3. Neurite outgrowth from these cells was inhibited by MAG (Fig. 5[a]A). As with PC12 cells, treatment of retinal neurons cells with C3 allowed neurite extension on the growth inhibitory MAG substrates to an extent similar to that observed on control substrates (Figs. 5[b]B and [5]C).--

Paragraph beginning at page 28, line 8, has been amended as follows:

--To explore the possibility that treatment of damaged axons with C3 might foster regeneration *in vivo*, we examined regeneration of retinal ganglion cell (RGC) axons in the optic nerve 2 weeks after optic nerve crush. Recently, it has been shown that microlesions in the CNS reduce the extent of the glial scar and allow axons access to CNS white matter distal to the lesion (Davies, S.J.A., *et al.* (1997) *Nature* 390, 680-683). To make microlesions of optic nerve, 10.0 sutures were used to axotomize RGC axons by constriction (Fig. 7[a]A). Retrograde labeling of RGCs from the superior colliculus (not shown), as well as anterograde labeling techniques (eg., Fig 7[a]A) verified that RGC axons were effectively axotomized. To apply C3 to crushed nerves, Gelfoam soaked with 2 mg/ml C3 was wrapped around the left optic nerve at the crush site, and two Elvax tubes, each loaded with 20 mg of C3, were positioned for sustained slow release (Fig. 7[a]A). Twelve animals were treated with C3 and a further 8 animals were treated with PBS as controls. Crushed and regenerating axons were visualized by anterograde labeling with cholera toxin injected into the eye 12 days after optic nerve crush (Fig. 7[a]A). Fourteen days after optic nerve crush, longitudinal cryostat sections of the optic nerves were examined by fluorescent microscopy for immunoreactivity to cholera toxin to detect anterogradely labeled RGC axons.--

Paragraph beginning at page 28, line 23, has been amended as follows:

--In control optic nerves that received optic nerve crush alone, no RGC axons extended past the crush site (n= 3 animals). In control animals treated with PBS-Elvax pellets and gelfoam, the crush site was easily detected where most anterogradely labeled axons stopped abruptly (Fig. 7[c]C). However, in these animals, a few axons did extend past the crush (Fig. 7[c]C, arrows), and the numbers of axons that regenerated varied from animal to animal. The application of Gelfoam and Elvax tubes may have altered the response to injury. Nonetheless, the response to C3 treatment applied with this lesion paradigm was dramatic.--

Paragraph beginning at page 29, line 4, has been amended as follows:

--We observed that C3 treatment allowed many RGC axons to grow past the region of the lesion. In 7 of 12 C3-treated animals, the lesion site was not clearly defined because of the large

numbers of axons that extended through the site (Figs. 7[d and e]D and E). Many of the axons that extended past the lesion site showed a twist path of growth, supporting their identification as regenerating axons (Fig. 7[f]F). A quantitative comparison of C3 and PBS treated animals revealed that more fibers grew past the lesion site after C3 treatment than after PBS treatment (Fig. 7[b]B). For this analysis we made a conservative estimate of the lesion site based on morphology, and counted the number of fibers in the distal optic nerve in 14 μ m sections. Seven of 12 C3-treated animals showed at least one section with 10-20 axons extending 250 μ m past the crush, compared with 1 of 8 of the PBS-treated controls (Fig. 7). In some animals regenerating axons were observed up to 1 mm from the crush, an extent of regeneration similar to that observed in mouse optic nerve after treatment with IN-1 antibody to block myelin inhibitors where fibers extended up to 750 μ m ([Bartsch, U., *et al.*, (1995) *Neuron* 15, 1375-1381).--

Paragraph beginning at page 29, line 18, has been amended as follows:

--Rats were anesthetized with 0.6 ml/kg hypnorm, 2.5 mg/kg diazepam and 35 mg/kg ketamin. The left optic nerve was exposed by a supraorbital approach, the optic nerve sheath slit longitudinally, the optic nerve lifted out and crushed 1 mm from the globe by constriction with a 10.0 suture held for 60 seconds (Fig. [4a]7A). For C3 treatment and buffer controls, Gelfoam soaked in PBS or 2mg/ml C3 transferase was placed on the nerve at the lesion site. Two 3 mm long tubes of Elvax (Sefton, *et al.*, (1984) loaded with buffer or 20 mg C3 were inserted in the Gelfoam near the nerve for continued slow release of C3 (Fig. [4b]7A). Twelve days after crush, 5 ml of 1% cholera toxin β subunit (List Biological laboratories, Inc., Cambell, CA) was injected into the vitreous to anterogradely label retinal ganglion cell axons (Fig. [4c]7A). Two weeks after optic nerve crush the animals were fixed by perfusion with 4% paraformaldehyde, and the eye with attached optic nerve was removed and postfixed in 4% paraformaldehyde. Longitudinal cryostat sections were processed for immunoreactivity to cholera toxin with goat anti-cholera toxin at 1:12,000 (List Biol. Labs Inc., CA) followed by rabbit anti-goat biotinylated antibody (1:200, Vector Labs, Burlingame, CA), and DTAF-streptavidin (1:500, Jackson Immunoresearch Laboratories).--

In the claims:

Claims 32 and 33 have been cancelled.

Claim 34 has been added.

--34. A method of suppressing the inhibition of neuronal axon growth, the method comprising delivering *Clostridium botulinum* ADP-ribosyl transferase directly at a central nervous system (CNS) lesion site in a patient or a peripheral nervous system (PNS) lesion site in a patient, wherein the *Clostridium botulinum* ADP-ribosyl transferase is delivered in an amount effective to suppress inhibition of neuronal axon growth.--

In the abstract:

The following abstract has been added:

--The invention provides a method of suppressing the inhibition of neuronal axon growth. The method involves delivering *Clostridium botulinum* ADP-ribosyl transferase directly at a nervous system legion site in a patient.--